cumstance, uptake of arginine and glycocyamine may be regulated by cellular requirements unrelated to the known metabolic synthetic reactions; output would reflect the circumstance where synthetic capacity of the kidney is more than adequate for the demand for phosphogens. An analogous situation is seen in the renal extraction of glucose, where under some circumstances output is observed, and under other circumstances uptake of arterial glucose occurs(14).

Summary. Renal extractions of arterial glycocyamine and arginine were determined by sampling urine and right renal venous blood in 25 males studied during diuresis because of hypertensive or urologic disease. Both positive and negative extractions of both compounds were observed. The finding of negative extractions is compatible with the capacity for renal synthesis of these compounds shown *in vitro* in other species. The finding of positive extractions is discussed in terms of utilization of these compounds in local renal metabolism, in addition to previously postulated roles as substrates for extrarenal syntheses.

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Immunologic, Chemical and Isotopic Identification of C^{14}-Labeled Bacterial Polysaccharide in Animal Tissues.* (27505)

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Upon parenteral injection polysaccharides isolated from bacteria have been found to remain for many weeks in animal tissues(1-5). Slow degradation of such polysaccharides in animal tissue has been suggested by studies with isotopically labeled products(4). After the first few days following intravenous injection of C^{14}-labeled Klebsiella polysaccharide, the label was excreted slowly in the urine while the C^{14} content of tissue gradually decreased(4). Although approximately 30% of the isotopic label in animal tissue was recovered by a simple extractive procedure and found to have some of the haptenic property of the injected labeled polysaccharide(4,6), the nature of most of the label in tissue remained uncertain. The present paper concerns an improved method of extraction from animal tissue, the chemical determination of monosaccharide constituents of Klebsiella polysaccharide and identification of the recovered material through relative isotopic labeling of the 3 constituent monosaccharides as well as by haptenic properties.

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Materials and methods. Capsulated *Klebsiella pneumoniae,* type B, were harvested from the surface of agar plates and the polysaccharide extracted with 0.1N NaOH, fol-

lowed by precipitation in 70% ethanol(4,7). The polysaccharide was labeled biosynthetically by addition of sodium acetate-1-C^{14} to the media and isotopic content of the extracted polysaccharide determined(4). Unlabeled and labeled polysaccharide samples were hydrolyzed with 2N HCl at 98°C for 3 hours and chromatography of hydrolysates carried out on Whatman No. 1 paper with 2 solvent systems: butanol-acetic acid-water (50 : 12 : 25) and pyridine-ethyl acetate-acetic acid-water (50 : 5 : 1 : 3)(8). The C^{14} in the chromatograms was localized and quantitated by a recording ratemeter and by counting serial 5 mm transverse strips of the chromatograms in a windowless flow counter. Chromatograms were sprayed with glucose oxidase(9), anisidine(10), aniline(11) and ninhydrin reagents. Rhamnose was further identified by absorption spectra in cysteine-H_2SO_4(12). Absorption spectra of aqueous solution of the original polysaccharide were determined from 210 to 600 mμ.

Labeled polysaccharide, prepared as a 1% solution in sterile, non-pyrogenic, 0.15M NaCl, was injected by tail vein, 1 mg polysaccharide per 100 g body weight, into male Holtzman rats, each weighing 150 to 170 g. Animals were killed 1 to 14 days later and liver, spleen and lymph nodes extracted with various sequences of ethanol, trichloracetic acid (TCA), papain, deoxyribonuclease and ribonuclease and hot 30% KOH. Usually, the last step in all sequences of extraction was the precipitation of polysaccharide with 70% ethanol containing 1% sodium acetate at 4°C for 12 hours, then recovery of the precipitate by centrifugation. The precipitate was redissolved in water and again precipitated with ethanol and sodium acetate. Papain, prepared by the method of Kimmel and Smith (13) was used with phosphate buffer pH 6.9 at different temperature ranges and time periods. Ribonuclease and deoxyribonuclease in acetate buffer pH 5.0 were used at 37°C for periods up to 6 hours. After each enzyme treatment the material was dialyzed at 4°C against 1 l of the same buffer with 3 daily changes. Ribonucleic acid (RNA) was determined by orcinol technic, corrected for DNA(14), deoxyribonucleic acid (DNA) by diphenylamine(15) and carbohydrate by the anthrone procedure(16) using glucose as a standard. Protein was estimated by the biuret method(17).

Tissues from normal rats not injected with polysaccharide were also homogenized, labeled polysaccharide added and extractive procedures carried out as indicated above. The labeled material recovered from the rat tissue was dissolved in 0.15M NaCl, clarified by centrifugation at 20,000 $\times$ *g*, 4°C, 30 minutes, and mixed with 0.15M NaCl dilutions of similarly clarified rabbit anti-Klebsiella sera as well as control rabbit sera(18). After 12 hours at 4°C the mixtures of tissue extract and sera were again centrifuged, the supernatant carefully decanted, the tube drained in an inverted position, and isotopic content determined in supernatant and precipitate after dissolving the latter in .01N NaOH. Another portion of the labeled tissue extract was hydrolyzed, chromatography carried out, and isotopic content localized as indicated above.

Results. The polysaccharide extracted from *Klebsiella pneumoniae,* type B, consisted of 3 monosaccharides-glucose, glucuronic acid and rhamnose (Table I). Paper chromatography of the unlabeled polysaccharide, hydrolyzed at 98°C for 3 hours in 2N HCl, disclosed glucose, glucuronic acid, glucuronolactone and a zone with the same R_f value as rhamnose. The presence of glucose was verified by spraying an alternate paper strip with glucose-oxidase reagent. In the cysteine-H_2SO_4 reaction the absorption spectra of the polysaccharide were typical for a methyl pentose. Ninhydrin-positive areas in the paper chromatograms were negligible. Nucleic acid was excluded by the absence of absorption at 260 mμ in aqueous solution of the original polysaccharide. The reaction for organic phosphate was negative. The C^{14}-labeled polysaccharide, hydrolyzed and chromatographed under the same conditions as for the unlabeled material, showed C^{14} in the 3 component sugars (Table I). Glucuronic acid had the lowest relative incorporation of isotope.

The most satisfactory recovery of labeled polysaccharide from tissue was attained by initial proteolysis with papain, then removal

TABLE I. Chemical Composition of Klebsiella Polysaccharide, and Comparison of Relative Isotopic Labeling and Haptenic Property of Injected with Extracted Polysaccharide.

	Composition (%)						Isotopic precipitin*	
	Glucuronic acid(24)		Glucose		Rhamnose		Klebsiella antisera	Control sera
	Wt	C^{14}	Wt	C^{14}	Wt	C^{14}		
Original polysaccharide	25.0	11.5	55.0	61.5	16.5	27.0	78.0–99.0	.5–2.0
Liver extract		—		59.3		24.8	56.5	5.5
Mesent. lymph node extract		7.0		72.6		20.2	75.0	2.5
Spleen extract		—		59.0		18.5	65.0	3.0

* % C^{14} in precipitate; 2 to 10 μg polysaccharide C^{14} (equivalent)/ml AB.

of RNA and DNA (either with or without TCA precipitation) by their respective nucleases followed by precipitation of polysaccharide in the non-dialyzable fraction with 70% ethanol containing 1% sodium acetate. By this procedure approximately 80% of the C^{14} in the liver, spleen and lymph nodes was recovered free of lipid, protein and nucleic acids. However, the admixture of recovered polysaccharide with an endogenous carbohydrate, especially liver glycogen, resulted in C^{14}/g value lower than that of the original injected bacterial polysaccharide. Chromatography of the hydrolysates of the labeled material extracted from tissue disclosed relative isotopic labeling of glucose and rhamnose similar to that of the original material (Table I), but label could not be identified by the glucuronic acid or glucuronolactone areas of liver and spleen extracts. In the chromatograms a relative small amount of C^{14} was found in a few areas other than those for the 3 constituent monosaccharides of the Klebsiella polysaccharide. Most of the labeled material recovered from animal tissues precipitated with anti-Klebsiella rabbit sera while traces precipitated with control rabbit sera.

Comments. The polysaccharide extracted from the capsulated *Klebsiella pneumoniae* was comprised of glucose, glucuronic acid and rhamnose. The capsular polysaccharide of type II pneumococcus has been found to consist also of these 3 monosaccharides(19), thereby affording a chemical basis for the marked immunologic similarity of a Klebsiella and type II pneumococcus reported by Avery, Heidelberger and Goebel(20) in 1925. These authors observed the high glucose content in the 2 polysaccharides. The arrangement of the 3 monosaccharides in these macromolecular polysaccharides remains unknown. Glucose and glucuronic acid were common in polysaccharides of the Klebsiella-Aerogenes microorganisms, but a methyl pentose constituent, when present, was usually fucose, not rhamnose(4). The abundance of the Klebsiella polysaccharide and the ease of extraction with alkali, warm water or warm phenol (unpublished studies) supported the opinion of Avery, *et al.*(20) that the material was of capsular origin.

The toxicity of lipopolysaccharide endotoxins of gram-negative bacteria has been attributed to the lipid component(21) but the latter alone has only a fraction of the biologic activity of the complex(22). The many biologic actions of the present lipid-free Klebsiella polysaccharide supported the suggestion that the toxic property of a lipopolysaccharide resided in the polysaccharide component, especially that of high molecular weight(23).

Following injection of bacterial capsular polysaccharides, the morphologic and functional manifestations of toxicity in surviving animals disappeared within hours or a few days(4) while the haptenic property, through which such substances were identified, remained in animal tissue for many weeks. Thus, the toxic properties were not dependent upon antigenic structure or monosaccharide composition.

The gradual loss of isotope from tissue, after uptake of parenterally introduced C^{14}-labeled Klebsiella polysaccharide, and the continued urinary loss of C^{14} moieties of low molecular weight(4) indicated the degradation of the polysaccharide within tissue. There are several possible explanations for the apparent decrease in or lack of glucuronic acid

in the still-haptenic polysaccharide extracted from tissue: a) the low level of isotope in this moiety, b) the carboxylation in tissue, especially if a major portion of C^{14} were in this portion of the monosaccharide, or c) artifacts resulting from hydrolysis of minute quantities of the polysaccharide admixed in extracts with other tissue constituents, d) tissue degradation of glucuronic acid and lack of dependence of haptenic property upon this constituent. The absence of detectable glucuronic acid in some tissue extracts was not incompatible with their retention of haptenic property. The anti-serum was produced by the whole Klebsiella organism. If the capsular polysaccharide, antigenic only in combination with other microorganismal constituents, was slowly degraded in the animal tissues, then antibody might be produced against such modifications of the polysaccharide. Such an explanation would be compatible with immunologic (or haptenic) specificity, some cross-reactions among bacterial polysaccharides, and the present observations on labeled glucuronic acid moiety in tissue. The apparently greater affinity of polysaccharide for nucleic acid *in vivo* than *in vitro*, observed during the various extraction procedures, may have some role in immunologic paralysis produced by haptenic bacterial polysaccharides.

Summary. Polysaccharide isolated from encapsulated *Klebsiella pneumoniae*, type B, has been found to consist of glucose, glucuronic acid and rhamnose. C^{14} was incorporated biosynthetically into these 3 components from acetate-1-C^{14} added to the media. Following injection of the C^{14}-labeled polysaccharide into animals, up to 80% of the labeled material has been recovered from animal tissue by a combination of enzymatic and extractive procedures. Much of the C^{14}-labeled material in tissue retained its original haptenic properties and relative C^{14} content of the constituent monosaccharides.

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